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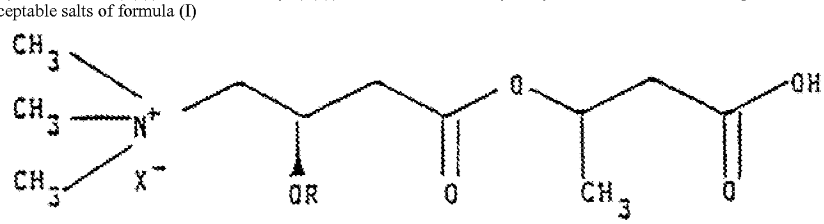
(56) References cited: :

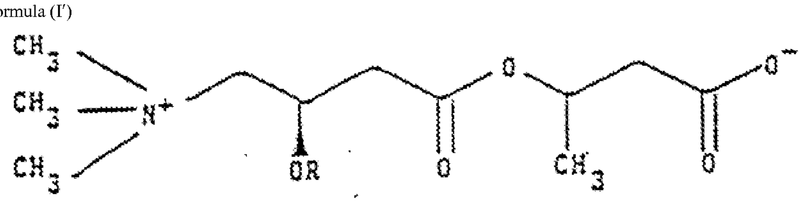
(54) Esters of (R)(-)-carnitine and acyl (R)(-)-carnitines with beta-hydroxybutyric acid and pharmaceutical compositions containing them for inhibiting neuronal degeneration, liver proteolysis and for the treatment of coma

(57) The esters of (R)(-)-carnitine and acyl (R)(-)-carnitines with beta-hydroxybutiric acid in the form of pharmacologically acceptable salts of formula (I)



(I)

wherein X is the anion of a pharmacologically acceptable salt, e.g. chloride, bromide, orotate, acid aspartate, acid citrate, acid phosphate, acid fumarate, lactate, acid maleate, acid oxalate, acid sulfate and glucosephosphate or in the form of inner salts of formula (I′)

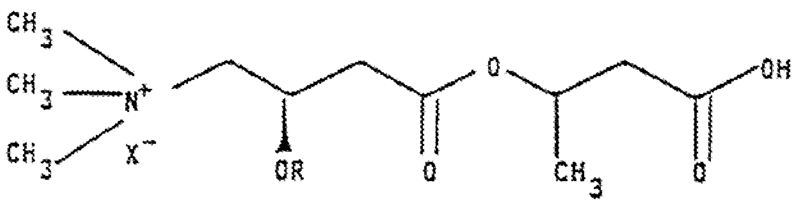


(I′)

wherein R is hydrogen or a straight or branched acyl group having from 2 to 5 carbon atoms, such as e.g. acetyl, propionyl, n-butyryl, isobutyryl and isovaleryl, are active in inhibiting neuronal degeneration (as it occurs in Alzheimer's senile dementia and Parkinson's disease), liver proteolysis and in the treatment of coma.

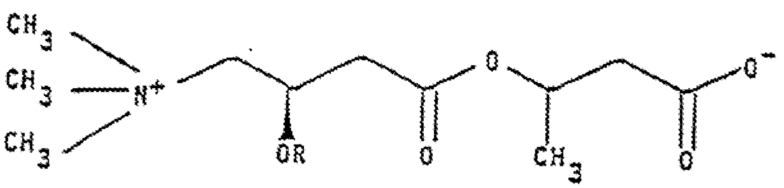
Description

[0001] The present invention relates to the esters of R(-)-carnitine and acyl (R) (-)-carnitines with beta-hydroxybutyric acid in the form of their pharmacologically acceptable salts of formula (I)



(I)

wherein X is the anion of a pharmacologically acceptable acid e.g. chloride, bromide, orotate, acid aspartate, acid citrate, acid phosphate, acid fumarate, lactate, acid maleate, acid oxalate, acid sulfate and glucosephosphate, or in the form of inner salts of formula (I')



(I')

wherein R is a hydrogen or a straight or branched acyl group having from 2 to 5 carbon atoms, such as for instance acetyl, propionyl, n-butyryl, isobutyryl and isovaleryl.

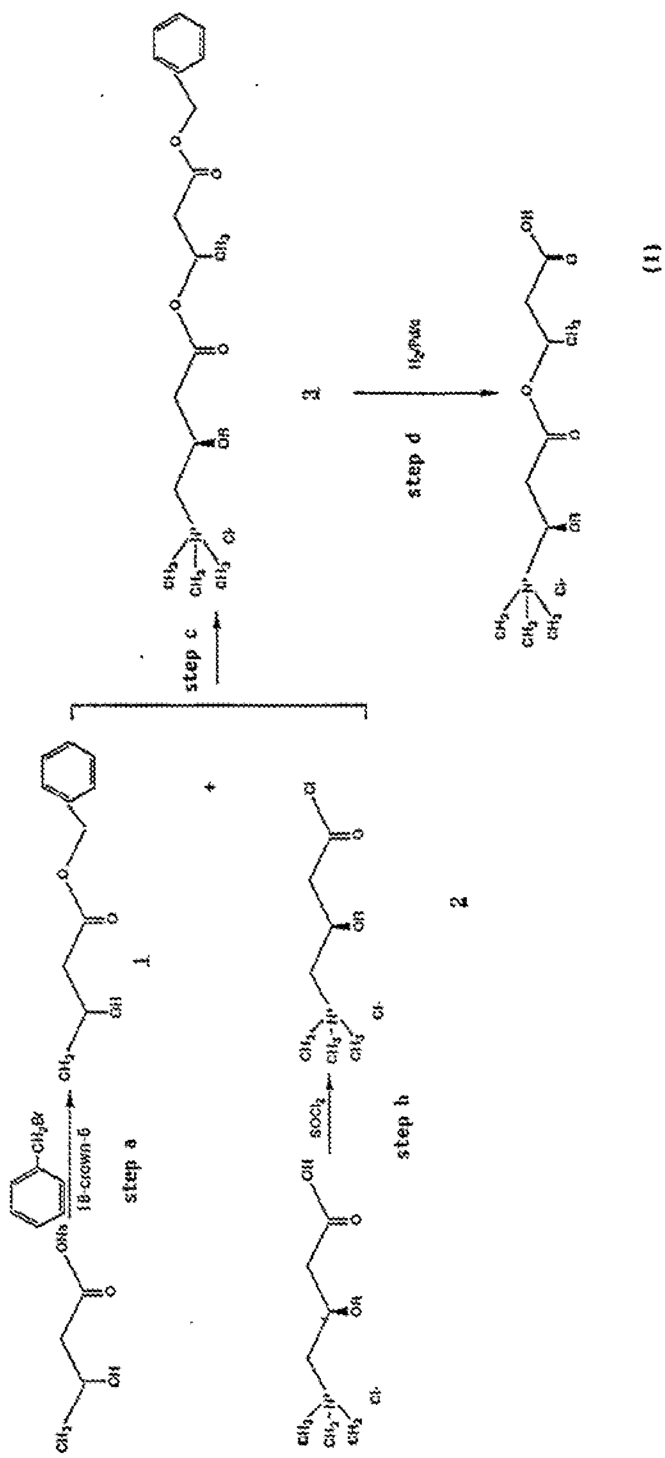
[0002] These compounds are active in inhibiting neuronal degeneration (as it occurs in Alzheimer's senile dementia and Parkinson's disease) and liver proteolysis and in the treatment of coma.

[0003] The present invention also relates to orally or parenterally admnistrable pharmaceutical compositions for treating the foregoing pathologies, which comprise one of the compounds of formula (I) or (I') as active principle.

[0004] Esters of carnitine with hydroxy-substituted saturated organic acids (e.g. 2-hydroxybutyric, 2-hydroxy-2-methylbutyric and 2-methyl-3-hydroxy propionic acid) are known already; see e.g. US patent 4,766,222 assigned to Sigma-Tau Industrie Farmaceutiche Riunite S.p.A. These compounds, however, are O-esters (i.e. esters on the carnitine hydroxyl group) and endowed with pharmacological properties entirely different from and in no way related to the properties of the esters of the present invention.

[0005] Esters on the carnitine carboxyl group are described in Z. Physiol. Chem., 295, 377, 1953 and Z. Physiol. Chem., 346, 314, 1966. These are, however, esters of carnitine with aliphatic alcohols such as methanol, ethanol and butanol or with aromatic alcohols such as benzyl alcohol, not with hydroxy-acids.

[0006] The non-limiting examples that follow show the preparation of the esters of acyl (R)(-)-carnitine chloride with beta-hydroxybutyric acid via the synthesis scheme which is illustrated hereinbelow.



Example 1

[0007] Preparation of the ester of isovaleryl (R)(-)-carnitine chloride with (R,S)(±)-beta-hydroxybutyric acid (ST 687).

Step a:

[0008] Preparation of the benzyl ester of (R,S)(±)-beta-hydroxybutyric acid 1 (R,S)(±)-beta-hydroxybutyric acid sodium salt (1.2 g; 0.01 moles) was suspended in benzyl bromide (6 ml; 0.05 moles).

[0009] 18 crown - 6 (0.264 g) dissolved in 7 ml acetonitrile was added to the mixture.

[0010] The solution was partially concentrated under a nitrogen stream and then kept under stirring at 80°C for 90 minutes. Following cooling, hexane - H_2O was added. The separated and dried organic phase was concentrated and then distilled under vacuum to remove the excess of benzyl bromide.

[0011] A solid residue (1.1 g) was obtained which was identified as the title product, yield 56%, TLC $CHCl_3$9 - MetOH 1
Rf = 0.8
Gas chromatography column HP_1 25 m; 0.32 mm ID; 0.33 μm film
thickness
carrier (He) flow rate: 1 ml/min
Make up gas 40 ml/min
Splitting ratio 40 ml/min
Injector 220°C
Detector(Fid) 280°C
T column 120°C for 3 minutes, 15°C/min 250°C
Rt = 9.36 product 1
Rt = 4.84 benzylbromide absent
NMR $CDCl_3$ δ7.3(5H,s,benzyl); 5.2(2H,s,CH_2-benzyl); 4.2(1 H,m,CH);
2.8(1 H,s,broad OH); 2.5(2H,d,-CH_2COO); 1.2(3H,d,CH_3)

Step b:

[0012] Preparation of the acid chloride of isovaleryl R(-)-carnitine chloride 2 Thionyl chloride (7.7 ml; 0.1 moles) was added to isovaleryl (R)(-)-carnitine chloride (10 g; 0.035 moles).

[0013] The resulting mixture was kept at room temperature for 4 hours, then concentrated under vacuum to remove the excess of thionyl chloride. The residue was washed 3 times with anhydrous ethyl ether. The reaction raw product thus obtained was used in the following step without further purification.

Step c:

[0014] Preparation of the ester of isovaleryl (R)(-)-carnitine chloride with (R,S)(±) beta-hydroxybutyric acid benzyl ester 3.

[0015] Acid chloride of isovaleryl (R)(-)-carnitine chloride of step b (0.035 moles) was dissolved in 25 ml anhydrous tetrahydrofurane.

[0016] (R,S)(±) beta-hydroxybutyric acid benzyl ester (7 g; 0.035 moles) of step a was added to the solution.

[0017] The resulting reaction mixture was kept at 25°C under stirring overnight, then ethyl ether was added till complete precipitation. The solid product thus obtained was filtered and washed with ethyl ether. 14 g of product 3 were obtained. Yield 89%.

NMR D_2O δ 5.7(5H,m,benzyl); 5.5(1H,m,-CH);

5.2(1H,m,COOCH); 5.0(2H,s,CH_2-benz.)

3.8(2H,m,NCH_2); 3.2(9H,s,$(CH_3)_3N+$);

2.8-2.5(4H,dd,$\underline{CH_2}$-COOCH;CH_2COO) 2.2(2H,d,$OCOCH_2$)

1.8(1H,m, $\underline{CH}\begin{smallmatrix}\diagup CH_3\\ \diagdown CH_3\end{smallmatrix}$); 1.2(3H,d,CH-$\underline{CH_3}$); 0.8(6H,d,$CH\begin{smallmatrix}\diagup \underline{CH_3}\\ \diagdown \underline{CH_3}\end{smallmatrix}$)

Step d:

[0018] Preparation of the ester of isovaleryl (R)(-)-carnitine chloride with (R,S)(±)-beta hydroxybutyric acid.

[0019] The product of step c (14 g; 0.031 moles) was dissolved in H_2O - ethanol (1:1) (1000 ml) and then hydrogenated in the presence of 1.5 g 10% Pd/C at the pressure of 4 atmospheres for 2 hours.

[0020] The reaction mixture was filtered, concentrated to dryness under vacuum and the residue was crystallized from acetone-ethyl ether giving 10 g of a hygroscopic product.
TLC chloroform 4.2 Isopr OH 0.7 MeOH 2.8 H_2O 1
AcOH 1.1 R_F: 0.7
$[\alpha]_D^{25}$ = -21(c = 1, H_2O).

NMR D_2O δ 5.7 (1H,m,$\underset{\substack{|\\ OCO}}{CH}$); 5.3 (1H,m,-COOCH-);

3.8(2H,m,N^+CH_2); 3.2 (9H,s,$(CH_3)_3N^+$);

2.8-(2H,d,CH_2-COO); 2.6(2H,d,CH_2COOH)

2.2(2H,d,$OCOCH_2$); 1.8(1H,m,$\underline{CH}\begin{smallmatrix}\diagup CH_3\\ \diagdown CH_3\end{smallmatrix}$); 1.2(3H,d,CH-$\underline{CH_3}$);

0.8(6H,d,$CH\begin{smallmatrix}\diagup \underline{CH_3}\\ \diagdown \underline{CH_3}\end{smallmatrix}$)

HPLC
Column µBondapack - C_1
Eluant KH_2PO_4 0.05 M - CH_3CN (85 -15)
UV detector λ = 205

Flowrate 1ml/min
Rt = 14-16 (the diastereomers are shown)

E.A. = $C_{15}H_{30}NO_6Cl$		C	H	N
	calc.	50.6	8.4	3.9
	found	48.93	8.36	3.49

Example 2

[0021] Preparation of the ester of isobutyryl (R)(-)-carnitine chloride with (R,S)(±)beta-hydroxybutyric acid (ST 730)

Step a:

[0022] same as in Example 1

Step b:

[0023] same as in Example 1, except that isovaleryl (R)(-)-carnitine chloride was substituted by isobutyryl (R)(-)-carnitine chloride

Step c:

[0024] The intermediate 3, ester of isobutyryl (R)(-)-carnitine chloride with (R,S)(±)-beta-hydroxybutyric acid benzyl ester was purified via δ prep 300 preparative HPLC.
Column prepak C_1
Eluant H_2O-CH_3CN 70-30
Flowrate 20 ml/min
Yield 50%

NMR D_2O δ 7.5(5H,s,aromatic); 5.8(1H,m,-CH(OCO)-); 5.3(m,1H,COOCH(CH₃)); 5.1(2H,s,CH_2benz.); 4.0-3.8(2H,m,$\overset{+}{N}CH_2$); 3.2(9H,s($CH_3)_3\overset{+}{N}$); 2.8(2H,m, CH_2COO); 2.6(2H,m,CH_2COOH); 1.8(1H,m,OCOCH); 1.3(3H,d,CH-CH_3); 1.2(6H,d,C(CH_3)(CH_3))

analytic HPLC
Column μ Bondapak C_1
Eluant phosphate buffer 0.05M-CH_3CN 60-40
Flowrate 1 ml/min UV detector λ = 205 nm
Rt = 10.75

Step d

[0025] Ester of isobutyryl-(R)(-)-carnitine with (R,S)(±)beta-hydroxybutyric acid (ST 730).

[0026] Same as step d of Example 1.

$[\alpha]_{D}^{25} = -20.3 (C = 1 H_2O)$.

TLC $CHCl_3 - H_2O$-IsopOH - MeOH -AcOH

(4.2 - 1.05 - 0.7 - 2.8 - 1.05)

NMR D_2O δ 5.7 (1H,m,-CH(OCO)-); 5.25(1H,m,COOCH(CH₃)); 3.9-3.7(2H,m,N^+CH_2);

3.2(9H,s$(CH_3)_3N^+$); 2.9(1H,m,CH_2COO); 2.7(2H,m,CH_2COOH);

1.9(1H,m,OCOCH); 1.3(3H,d,CH-CH_3); 1.1(6H,d,$CH(CH_3)_2$)

$C_{14}H_{28}NO_6Cl$		C	H	N	Cl
	calc.	49.19	8.25	4.10	10.04
	found	50.26	8.12	3.59	10.61

HPLC
Column μ Bondapack - C_1
Eluant KH_2PO_4 0.05M - CH_3CN 85-15
UV detector λ = 205 nm
Flowrate 1 ml/min
Rt = 8.10 - 9.98 (the two diastereomers are thus shown)

Example 3

[0027] Preparation of the ester of acetyl (R)(-)-carnitine chloride with (R,S)(±)beta-hydroxybutyric acid (ST 765)

Step a:

[0028] same as in Example 1

Step b:

[0029] same as in Example 1, except that isovaleryl (R)(-)-carnitine chloride was substituted by acetyl (R)(-)-carnitine chloride.

Step c:

[0030] Intermediate 3, ester of acetyl (R)(-)carnitine chloride with (R,S)(±)-beta-hydroxybutyric acid benzyl ester, was purified via preparative HPLC as described in step C of Example 2.
Yield 50%

NMR D_2O δ 7.5(5H,s,aromatic); 5.7(1H,m,-CH-); 5.4-5.0(3H,m,s,COOCH-,
OCO
CH_2-Ar); 3.8(2H,m,N^+CH_2); 3.2(9H,s,$(CH_3)_3N^+$); 2.8-2.5(4H,m,$COOCH_2$,
CH_2COOH) 2.2(3H,s,$COCH_3$); 1.4(3H,d,$CHCH_3$)

analytic HPLC
Column μ Bondapack C_1
Eluarit phosphate buffer KH_2PO_4 0.05M - CH_3CN 60-40
Flowrate 1 ml/min
UV detector λ = 205 nm
Rt = 11.73

Step d:

[0031] Ester of acetyl (R)(-)-carnitine chloride with (R,S)(±)beta-hydroxybutyric acid (ST 765)

[0032] Prepared as described in step d of Example 1
$[\alpha]^{25}_{D}$ = -22.9 (H_2O 1.2%)
TLC $CHCl_3$ - H_2O - isoprOH - MetOH - AcOH (4.2 - 1.05 - 0.7 - 2.8 - 1.05)
Rf = 0.6

NMR D_2O δ 5.7(1H,m,-CH-); 5.3(1H,m,COOCH); 3.9-3.7(2H,m,N^+-CH_2);
OCO
3.2(9H,s,$(CH_3)_3N^+$); 2.9(2H,m,CH_2COO); 2.7(2H,m,CH_2COOH)
2.2(3H,s,$COCH_3$); 1.4(3H,d,$CHCH_3$)

E.A. $C_{13}H_{24}NO_6Cl$		C	H	N	Cl
	calc.	47.90	7.42	4.29	10.88
	found	47.14	7.57	4.88	10.64

H_2O 0.46%
HPLC
Column μ Bondapack C_1
Eluant phosphate buffer KH_2PO_4 0.05M - CH_3CN 90 - 10
Flowrate 0.5 ml/min
UV detector λ = 205 nm

Rt = 11.68 - 12.83 (the two diastereomers are thus shown).

Example 4

[0033] Preparation of the ester of propionyl (R)(-)-carnitine chloride with (R,S)(±)beta-hydroxybutyric acid (ST 780).

Step a:

[0034] same as in Example 1

Step b:

[0035] same as in Example 1, except that isovaleryl R(-)carnitine chloride was substituted by propionyl R(-)carnitine chloride.

Step c:

[0036] Intermediate 3, ester of propionyl (R)(-)-carnitine chloride with (R,S)(±)-beta-hydroxybutyric acid benzyl ester, was purified via preparative HPLC as described in step c of example 2.

Yield 50%

NMR $CDCl_3$ δ 7.3(5H,s,aromatic); 5.6(1H,m,-CH- (OCO)); 5.3(1H,m,CH (CH3));

5.1(2H,s,CH_2-Ar);4.0(2H,m,N^+CH_2); 3.4(3H,s,$(CH_3)_3N^+$); 2.9-2.5(4H,mCH_2CO

OCH; CH_2COOH); 2.3(2H,t,OCOCH_2); 1.4-1.0(6H,m,CH_2CH_3;CH_3CH)

analytic HPLC
Column μ Bondapack C_1
Eluant phosphate buffer 0.005 M 60
Acetonitrile 40
Flowrate 1 ml/min
UV detector λ = 205 nm
Rt = 8.46

Step d:

[0037] Ester of propionyl-(R)(-)-carnitine chloride with (R,S)(±)beta-hydroxybutyric acid (ST 780).

[0038] Prepared as described in step d of Example 1.
$[\alpha]^{25}_{D}$ = -23.9 (C = 1% H_2O)

NMR D_2O δ 5.6(1H,m,CH); 5.3(1H,q,CH); 3.8(2H,m,N^+CH_2); 3.2(9H,s,$(CH_3)_3$

OCO CH3

N^+); 2.9-2.4 (4H,d,d,$\underline{CH_2}$COOCH;$\underline{CH_2}$COOH); 1.3(3H,t,$CH_2\underline{CH_3}$); 1.0(3H,d,CH)

HPLC
Column μ Bondapack C_1
Eluant phosphate buffer KH_2PO_4 0.005 M 90
CH_3CN 10
Flowrate 0.5 ml/min
UV detector λ = 20.5 nm
Rt = 6.40-7.07 (the two diastereomers are thus shown).

Example 5

[0039] Preparation of the ester of R(-)-carnitine chloride with R,S(±)beta-hydroxybutyric acid (ST 784).

[0040] The compound was prepared as described in the previous Examples 1-4.
$[\alpha]_D^{25}$ = -11.1 (C = 1% H_2O)

NMR D_2O δ 5.3(1H,m,COOCH); 4.6(1H,m,$\underline{CH}$); 3.4(2H,dd,N^+CH_2);

OH

3.2(9H,s,$(CH_3)_3N^+$); 2.7(4H,m,$\underline{CH_2}$COOCH; $\underline{CH_2}$COOH);

1.3 (3H,d,CH-$\underline{CH_3}$)

HPLC
Column Novapak C_1
mobile phase $KH_2PO_4$50 mM
Flowrate 1 ml/min
Rt = 4.56-5.01 min (the two diastereomers are thus shown)

Example 6

[0041] Ester of isobutyryl (R)(-)-carnitine chloride with R(-)-beta-hydroxybutyric acid (ST 863).

[0042] The compound was prepared as described in Example 2 (ST 730)

[0043] The compound of step c, ester of isobutyryl (R)(-)-carnitine with R(-)-beta-hydroxybutyric acid benzyl ester, showed the following characteristics:
$[\alpha]_D^{25}$ = - 11.1 (C = 1% MetOH)
HPLC
Column μ Bondapack C_1
mobile phase $NaClO_4$0.05M-CH_3CN (60-40)

Flowrate 1.5 ml/min
UV detector λ = 205 nm
R_t = 15.64 min

[0044] The compound of step d, i.e. the title compound ester of isobutyryl (R)(-)-carnitine chloride with R(-)-beta-hydroxybutyric acid (ST 863), showed the following characteristics:
[α]
$^{25}_{D}$
= -11.6 (C = 1% H_2O)
HPLC
Column μ Bondapack C_1
mobile phase $KH_2PO_4$0.05M - CH_3CN 70-30
Flowrate 1 ml/min
UV detector λ = 205 nm
R_t = 8.25

Example 7

[0045] Ester of isobutyryl (R)(-)-carnitine chloride with S(+)-beta-hydroxybutyric acid (ST 864).

[0046] The compound was prepared as described in Example 2 (ST 730)

[0047] The compound of step c, ester of isobutyryl (R)(-)-carnitine chloride with S(+)-beta-hydroxybutyric acid benzyl ester, showed the following characteristics:
[α]
$^{25}_{D}$
= -15.4 (C = 1% MetOH)
HPLC
Column μ Bondapak C_1
mobile phase $NaClO_4$0.05M-CH_3CN (60-40)
Flowrate 1.5 ml/min
UV detector λ = 205 nm
Rt = 14.79 min

[0048] The compound of step d, i.e. the title compound ester of isobutyryl (R)(-)-carnitine with S(+)-beta-hydroxybutyric acid (ST 864), showed the following characteristics:
[a]
$^{25}_{D}$
= - 21.7 (C = 1% H_2O)
HPLC
Column μ Bondapack C_1
mobile phase $KH_2PO_4$0.05M-CH_3CN (70-30)
Flowrate 1 ml/min
UV detector λ = 205 nm
R_t = 7.32 min

Example 8

[0049] Ester of butyryl (R)(-)-carnitine chloride with (R,S)(±)-betahydroxybutyric acid (ST 877).

[0050] The compound was prepared as described in Example 1.

[0051] The compound of step c, ester of butyryl (R)(-)-carnitine chloride with (R,S)(±)-betahydroxybutyric acid benzyl ester, showed the following characteristics:
[α]
$^{25}_{D}$

= -12.8 (C = 1% H_2O)
HPLC
Column 53 ODS1 (100 mm x 1 mm) Sperisorb
mobile phase $KH_2PO_4$0.05M - CH_3CN 70-30
UV detector λ = 205 nm
Flowrate 0.1 ml/min
Rt = 30 min

NMR D_2O δ 7.5(5H,s,benzyl); 5.6(1H,m,CH);
OCO
5.2(3H,s+m,CH_2-benzyl; CH-CH_3);
3.7(2H,m,N^+CH_2-); 3.3(9H,s,$(CH_3)_3N^+$-);
2.8(4H,m,CH_2COO,$OCOCH_2$); 2.4(2H,t,CH_2COOCH_2);
1.7(2H,q,CH_2CH_3); 1.2(3H,d,$CHCH_3$);
1.0(3H,t,CH_2CH_3)

[0052] The compound of step d, i.e. the title compound ester of butyryl (R)(-)-carnitine chloride with (R,S)(±)-beta-hydroxybutyric acid (ST 877), showed the following characteristics:
$[\alpha]^{25}_{D}$
= -18.9 (C = 1% H_2O)
HPLC
Column Bondapak NH2
mobile phase $KH_2PO_4$0.05M - CH_3CN 35-65
UV detector λ = 205 nm
Flowrate 0.1 ml/min
Rt = 5.62

NMR D_2O δ 5.6(1H,m CH-); 5.2(1H,m,CH); 3.8(2H,m,N^+CH_2)
OCO CH_3
3.2(9H,s,$(CH_3)_3N^+$); 2.8(4H,m,CH_2COO,$OCOCH_2$);
2.4(2H,t,CH_2COOH); 1.7(2H,m,CH_2CH_3);
1.2(3H,d,$CHCH_3$); 1.0(3H,t,CH_2CH_3)

Effect of ST 687 on the neurologic deficit, memory impairment and cerebral oedema in post-oligaemic rats.

[0053] The study was conducted with a view to assessing the therapeutical effect of ST 687 administered i.p. to rats immediately after the effect of a transient forebrain oligohaemia in the experiment animals were detected. In particular, the propensity of post-oligaemic rats to develop conditioning was studied in a one-trial passive avoidance task. Concurrently, the neurologic deficit during a 3-day period following the ischaemic insult was assessed, and finally the extent of the oedema was assessed by measuring the water content of the cerebral tissue.

[0054] The effects of ST 687 were compared with those of acetyl (R)(-)-carnitine studied under identical experimental conditions.

[0055] In the experiments Sprague-Dawley (Iffa Credo) male rats weighing 230-250 g were used, that had been caged (5 rats/cage) under conditions of controlled temperature (22°C±1°C), 50% relative humidity and 12-hours dark-light cycle (light on from 8 a.m. to 8 p.m.). The rats were fed UAR (Epinay Orge, France) laboratory chow and had free access to tap water. The rats were caged for 5 days before surgery.

[0056] Under light ether anesthesia, carotid arteries were isolated and loosely surrounded by a thread. Twenty-four hours later, reversible incomplete forebrain ischemia was produced by bilateral common carotid artery occlusion combined with sodium nitroprusside-induced arterial hypotension (1.1 mg/rat s.c.). Mean arterial blood pressure (MABP) was lowered and maintained nearby 6.6 kPa for 45 min. Then it gruadually returned to normal within the 60th minute when carotid occlusion was removed.

[0057] The neurological deficit was assessed via the observational method described by Irwin S.: Comprehensive observational assessment: I a. A systematic, qualitative procedure for assessing the behavioural and physiologic states of the mouse. Psychopharmacologia (Berl.), 1968, 13 : 222-257, for quantifying the behavioural and physiologic state of the mouse. The rats were lifted vertically by mid-tail approximately 15 cm above a rod and lowered to elicit the visual placing response, usually characterized by an extension of forelimbs before contact. The rating was as follows: 3 = normal behaviour (the rat grasps the rod) : 2 = mild anterolateral rotation of the forelimbs (the grasping reflex only occurs when the rat is placed close to the rod) : 1 = severe rotation of the forelimbs and of the body (the grasping reflex occurs occasionally when the rat touches the rod) : 0 = no grasping reflex. The neurological deficit according to this criterion was evaluated respectively 3, 24, 48 and 72 hours following oligohaemia.

[0058] The functional aspects of the cerebral ischemic injury were assessed by a one trial learning procedure (passive avoidance reaction) as originally described by Kurtz K. H. e Pearl J.: The effects of prior fear experience in acquired drive learning. J. Comp. Physiol, Psychol, 1960, 53 : 201-206, and more extensively developed by Buresova et al.: Effect of atropine on learning, extinction, retention and retrieval in rats. Psychopharmacologia, 1964, 5: 255-263.

[0059] Four hours after clipping-off untreated post-oligaemic rats were placed into an apparatus consisting of a large illuminated compartment (40 x 40 cm) connected by an opening to a small dark compartment (10 x 10 cm) with an electrified grid floor. The animals placed into the large compartment were allowed to explore the apparatus for three minutes. The latency to enter and the time spent in the small compartment were measured with a stop watch. Habituation to the experimental conditions was repeated 24 and 29 hours after ischemia. At the end of the third habituation trial, the opening between the two compartments was closed and the rat, placed into the small compartment, received intermittent electrical foot-shocks for one minute. The retention of the passive avoidance towards the small compartment was tested 24 and 48 hours after the last habituation trial i.e. 53 and 77 hours post-oligohaemia, respectively. The criterion used to determine whether an animal was conditioned was based upon the rat remaining in the large illuminated compartment for 180 sec. without entry into the small dark compartment.

[0060] Immediately after the last retention trial, i.e. 77 hours post-oligohaemia, rats were sacrificed by decapitation, their brains rapidly removed and macroscopically examined in terms of swelling. Brain water content was determined by the method wet weight/dry weight.

Death-rate, cerebral oedema and passive avoidance in post-oligaemic rats and following treatment with acetyl(R)(-)-carnitine and ST 687

	Dose mg. kg i.p. twice a day	n.1	n.2	Death rate after 72 hours (%)	Incidence of cerebral oedema upon excision (3)	Incidence of retention of conditioned response (%)	
						+ 53 h	+ 77 h
Oligohaemia	0	19	9	52,6	66,6	44,4	33,3
Acetyl (R)(-)-carnitine	12,5	15	10	33,3	80,0	80,0	80,0
	25,0	17	10	41,2	60,0	60,0	70,0
	50,0	20	10	50,0	90,0	70,0	90,0
ST 687	2.5	19	10	47,4	60.0	20.0	40.0
	5.0	16	10	37,5	50,0	60,0	60.0
	7.5	16	6	62.5	66,7	53,3	66.7

n 1 = number of oligaemic rats

n 2 = number of survivors

n 3 = number of rats exhibiting cerebral oedema

$p < 0.05$ according to the continuity-corrected chi^2 test.

Protective effect of ST 784 against acetaminophen (paracetamol)-induced hepatic damage.

[0061] Paracetamol has been widely used as analgesic and antipyretic. Paracetamol overdosage is known to provoke serious hepatic damages.

[0062] Male Wistar rats weighing 200-250 g (15 rats/group) that had been keept fasting for at least 12 hours, were administered a single dose of paracetamol (1 g/kg body weight, per os). 100 g paracetamol were dissolved in 1000 ml of 5% (w/v) carboxymethylcellulose suspension in water. (Hence, the animals were actually administered 10 ml paracetamol solution/kg body weight). 101 mg ST 784/kg body weight were administered orally (as aqueous solution) 1, 8 and 24 hours, respectively, following paracetamol administration. The animals were sacrificed 32 hours following paracetamol administration.

[0063] Transaminases (SGOT and SGPT) were measured in blood serum. ST 784 provoked a decrease in transaminases exceding 60% ($p \leqq 5$) with respect to the control animals.

[0064] The compounds of the present invention are orally or parenterally administered, in any of the usual pharmaceutical forms which are prepared by conventional procedures well-known to those persons skilled in the pharmaceutical techonology. These forms include solid and liquid oral unit dosage forms such as tablets, capsules, solution, syrups and the like as well as injectable forms, such as sterile solutions for ampoules and phials.

[0065] For these pharmaceutical forms the usual solvents, diluents and excipients are used. Optionally, sweetening, flavouring and

preservative agents can also be bresent. Non limiting examples of such agents are sodium carboxymethylcellulose, polysorbate, mannitol, sorbitol, starch, avicel, talcum and other agents which will be apparent to those skilled in the pharmaceutical technology.

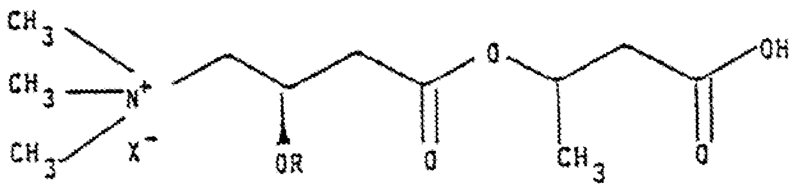
[0066] The dose which is administered will be determined by the attending physician having regard to the age, weight and general conditions of the patient, utilizing sound professional judgement. Although effective results can be noticed at doses as low as 5 to 8 mg/kg of body weight daily, a dose of from about 10 to about 50 mg/kg of body weight is preferred. Whenever necessary, larger doses can be safely administered in view of the low toxicity of the compounds of this invention.

[0067] As non-limiting examples and depending on the specific pharmaceutical form of administration, the following dosages can be indicated:
for the phials : from 5 to 500 mg
for the capsules : from 15 to 50 mg
for the tablets : from 15 to 500 mg
for the oral solution : from 15 to 50 mg

Claims

1. Esters of (R)(-)-carnitine and acyl (R)(-)-carnitines with beta-hydroxybutyric acid.

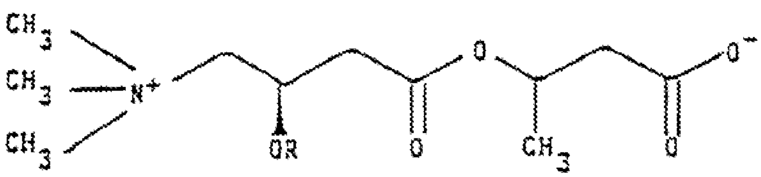
2. Esters according to claim 1, in the form of pharmacologically acceptable salts of formula (I)



(I)

wherein X is the anion of a pharmacologically acceptable acid and R is hydrogen or a straight of branched acyl group having from 2 to 5 carbon atoms.

3. Esters according to claim 1, in the form if inner salts (I')



(I')

4. Esters according to claims 2 or 3, wherein R is selected from hydrogen, acetyl, propionyl, n-butyryl, isobutyryl and isovaleryl.

5. Esters according to claim 2, wherein X is selected from chloride, bromide, orotate, acid aspartate, acid citrate, acid phosphate, acid fumarate, lactate, acid maleate, acid oxalate, acid sulfate and glucosephosphate.

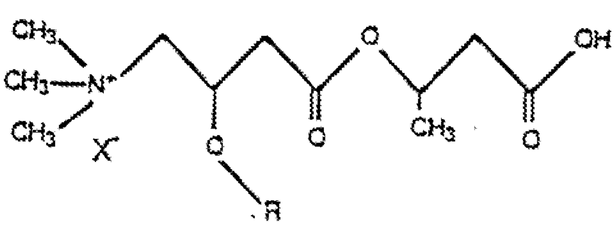
6. An orally or parenterally administrable composition comprising an ester of formula (I) or (I′) as active principle.

7. An orally or parenterally administrable composition for inhibiting neuronal degeneration, liver proteolysis and for the treatment of coma comprising an ester of formula (I) or (I′) as active principle and a pharmacologically acceptable excipient therefor.

8. Composition according to claim 7, in unit dosage form, comprising between about 5 and 500 mg of an ester of formula (I) or (I′)

Claims

1. A process for preparing esters of (R)(-)-carnitine and acyl (R)(-)-carnitine wiht β-hydroxybutyric acid of general formula (I)



(I)

wherein X is the anion of a pharmacologically acceptable acid and R is H or a straight or branched acyl group having from 2 to 5 carbon atoms, comprising:

1) condensing the sodium salt of β-hydroxybutyric acid with benzyl chloride in the presence of crown-ethers, in an organic solvent, in an inert gas atmosphere, at a temperature comprised between 20°C and 30°C, for 1 -2 hours, and isolating the benzylester of β-hydroxybutyric acid thus obtained via distillation under vacuum;

2) condensing the acid chloride of (R)(-)-carnitine or acyl (R)(-)-carnitine with the benzylester of β-hydroxybutyric acid in an inert anhydrous organic solvent, at a temperature comprised between 20°C and 30°C, for 12-24 hours, and isolating the compound thus obtained, acyl (R)(-)-carnitine ester with β-hydroxybutyric acid benzylester, from the reaction mixture by precipitation with an organic solvent, such as ethyl ether or acetonitrile; and
3) hydrogenating the compound obtained in step 2) in a water or ethanol solution or mixtures thereof, in the presence of a hydrogenation catalyst, such as 5% or 10% Pd/C, for 30-180 minutes, at a pressure of 2-5 hydrogen atmospheres, and isolating the product thus obtained, acyl (R)(-)-carnitine ester with β-hydroxybutyric acid, by concentrating under vacuum the solution to dryness.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 91 83 0062

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	DE-A-3015636 (SIGMA TAU S.P.A.) * the whole document *	1-8	C07C219/22 A61K31/22 A61K31/205
A D	GB-A-2071091 (SIGMA TAU S.P.A.) * the whole document * & US-A-4766222	1-8	
A	EP-A-167115 (MAGIS FARMACEUTICI S.P.A.) * the whole document *	1-8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C07C A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	25 APRIL 1991	RUFET, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
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